EXHIBIT 12

1: Exp Cell Res. 1997 Jun 15;233(2):288-96.

ELSEVIER FULL-TEXT ARTICLE

Characterization of cholesterol-free insect cells infectible by baculoviruses: effects of cholesterol on VSV fusion and infectivity and on cytotoxicity induced by influenza M2 protein.

Cleverley DZ, Geller HM, Lenard J.

Department of Physiology and Biophysics, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey 08854, USA.

The patented cell line from the cabbage looper Trichoplusia ni (High Five from Invitrogen) was found to grow readily under cholesterol-free (CF) culture conditions. Cellular cholesterol became undetectable by CF passage 4, while growth rate and overall cell morphology remained unaffected for at least 59 CF passages. The Golgi apparatus in CF cells was significantly smaller than in control cells, and the CF cells also concentrated a ceramide-based fluorescent Golgi marker to a greater extent, but endoplasmic reticulum morphology appeared unaffected. Two proteins were expressed in High Five cells from recombinant baculoviruses under CF and control conditions: the vesicular stomatitis virus (VSV) fusion glycoprotein G and the influenza virus ion channel M2. Both proteins were expressed in comparable amounts in CF and control cells. Both were properly assembled and transported to the plasma membrane in CF cells, indicating the presence of functional Golgi. Wild-type G protein expression resulted in extensive syncytia formation in both CF and control cells, showing that cholesterol is not required for VSV fusion. However, a mutant G protein lacking six transmembrane domain residues was inactive in both CF and control cells. Influenza M2 protein was functional in control cells, as indicated by its amantadine-inhibitable cytotoxicity, but cytotoxicity was absent in CF cells expressing this protein, indicating a cholesterol-dependence for the cytotoxic action of this protein. CF and control cells were both infectible with VSV. However, infected cell centers were modestly decreased (ca. 3.5-fold) in CF cells. CF cells offer a convenient and novel approach to the study of specific cholesterol functions.

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